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A New Species of the Genus *Tylototriton* (Amphibia, Salamandridae) from Hunan, China

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Abstract A new species of the genus *Tylototriton* is described in this article based on morphological and genetic evidence. The new species is identified belonging to the *T. asperrimus* group and is similar to *T. wenxianensis*. The diagnostic characters of the new species are as follows: dorsal ridge broad and thick, its width approximately equal to eye diameter ($P < 0.001$); tail height greater than width at base of tail ($P < 0.001$); no villous genital papilla found inside the male anal fissure; nodule-like warts, along lateral margin of the trunk, bulge and forming tubercles, and thin and transverse striae present between the tubercles.

Keywords new species, molecular phylogeny, taxonomy, Amphibia, Salamandridae, *Tylototriton*, Hunan, China

1. Introduction

The genus *Tylototriton* Anderson (1871) consists of nine species which are distributed over southern, eastern and southeastern Asia, including China, Vietnam, Laos, Nepal, Bhutan, Myanmar, Thailand, and India (Fei *et al.,* 2006; Böhme *et al.*, 2005; Stuart *et al.*, 2010). The nine species are classified into two clades: the *T. verrucosus* group and *T. asperrimus* group (Fei *et al.*, 2005, 2006), or two subgenera, the subgenus *Tylototriton* and subgenus *Yaotriton* (Dubois and Raffaëlli, 2009). The *T. verrucosus* group (= the subgenus *Tylototriton*; Dubois and Raffaëlli, 2009) includes four species: *T. kweichowensis* (Fang and Chang, 1932), *T. shanjing* (Nussbaum *et al.*, 1995), *T. taliangensis* (Liu, 1950), and *T. verrucosus* (Anderson, 1871). The *T. asperrimus* group (= the subgenus *Yaotriton*; Dubois and Raffaëlli, 2009) consists of five species: *T. asperrimus* (Unterstein, 1930), *T. hainanensis* (Fei *et al.*, 1984), *T. wenxianensis* (Fei *et al.*, 1984), *T. vietnamensis* (Böhme *et al.*, 2005), and *T. notialis* (Stuart *et al.*, 2010). Chen *et al.* (2010) described a new subspecies, *T. wenxianensis dabienicus*, based on the specimens collected from the Dabie Mountains in Henan, China, which were previously included in *T. wenxianensis* by Fei *et al.* (2005, 2006, 2010).

In the early 1980s, some specimens of the genus *Tylototriton* were collected in the Wuling Mountains in Sangzhi County, Hunan, China, and they were identified as *T. asperrimus* (Shen, 1989) based on the presence of "∧" shaped vomerine tooth rows, and a pair of obvious lateral bony ridges on the head reffering existed documents (Chang, 1936; Hu *et al.*, 1973, 1977; Tian and Jiang, 1986). The dorsal skin is extremely rough and covered with warts, the dense warts laterally on the trunk form knob-like warts, and the dorsum of the body and tail are completely black or blackish brown with no colored spots. Fei *et al.* (1984) found the new subspecies *T. asperrimus wenxianensis* in Wenxian County of Gansu, Pingwu County of Scihuan and Mt. Leigong of Guizhou, which was then upgraded to species rank, i. e., *T. wenxianensis* by Fei *et al.* (1990), and subsequently Fei *et al.* (2005, 2006, 2010) included the specimens collected from Sangzhi County and previously identified as *T. asperrimus* by Shen (1989) in the species *T. wenxianensis*.

Recently, we systematically studied the genus *Tylototriton*, including examination of the topotype of *T. asperrimus*, and the topotype and holotype of *T. wenxianensis*. The results confirmed that the specimens of

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T. wenxianensis from Sangzhi were similar to its topotype and holotype. However, distinct morphological and molecular genetic differences were found between the specimens from Sangzhi and other localities, as well as among other known species of the *T. asperrimus* group, supporting the argument that the Sangzhi population represents a previously unrecognized species.

2. Materials and Methods

2. 1 Specimens

Sangzhi population of *"Tylototriton wenxianensis"* : 35 ♂, 4 ♀ and 20 larvae were collected from the Tianping Mountains in Sangzhi County, northwestern Hunan, China.

Tylototriton asperrimus: 12♂: GXSD 82067001–82067007, GXSD 8206-7A–8206-7E, collected from the Dayao Mountains in Jinxiu County, Guangxi, China and preserved in the College of Life Sciences, Guangxi Normal University in Guangxi, China.

Tylototriton wenxianensis: CIB 638164 (holotype, ♂, collected in Wenxian County, Gansu, China), CIB 750041 (from Pingwu County, Sichuan, China), and CIB 750042–750046 (from Pingwu County, Sichuan, China, 5 larvae at Stage 46), preserved in the Chengdu Institute of Biology, Chinese Academy of Sciences in Sichuan, China. NWSD 090500a–b (from Qingchun County, Sichuan, 1 ♂, 1 ♀), preserved in the College of Life Sciences, Northwest Normal University in Gansu, China.

All these specimens are used for morphological comparisons, and morphological information on related species was obtained from related references (Fei *et al.*, 1984, 2006; Böhme *et al.*, 2005; Stuart *et al.*, 2010).

2.2 Statistical analysis Six basic measurements (total length = TOL, tail height = TH, width of basal tail = TW, width of dorsal ridge = DRW, eye diameter = ED, snout-vent length = SVL) were conducted on all specimens using a dial caliper. All these data were statistically analyzed using SPSS (V 12.0), and the Kruskal Wallis Test (KWT; nonparametric test) was used to test the differences among ratios. The significance level was set at $P < 0.05$. In addition, for the Sangzhi population, the following measurements were measured: head length = HL, head width = HW, head height = HH, snout length = SL, internasal space = INS, interorbital space = IOS, for limb length = FLL, hind limb length = HLL, space between axilla and groin = AGS, and tail length = TL.

2.3 Molecular systematic analyses

2.3.1 Sampling and gene sequencing A complete DNA sequence of the ND2 gene (1035 bp) from the mitochondria of nine samples was newly sequenced for this work (Table 1) and eight sequences of ND2 gene were downloaded from GenBank (Table 1). They were applied to represent 14 populations of the nine species in this work (Table 1, Figure 1), of which *Tylototriton taliangensis*, *T. kweichowensis* and *T. shanjing*were were used as outgroup (Table 1). Total DNA extraction, PCR (Polymerase chain reaction), and ND2 gene sequencing were carried out as described by Wang *et al.* (2009).

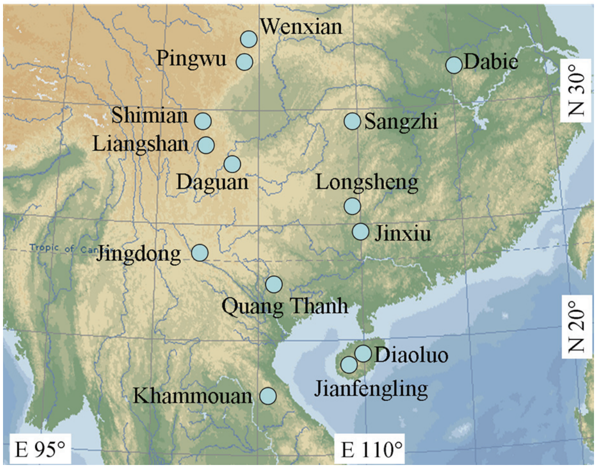

Figure 1 Sampling sites for genetic analysis in this work.

2.3.2 Reconstruction of phylogenetic tree and calculation of genetic distance All the sequences were aligned using ClustalX1.83 (Thompson *et al.*, 1997) with default settings. Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses were used to reconstruct the phylogenetic relationships. MP analysis was performed using PAUP 4.0b10 (Sworfford, 2002), the tree searching was a heuristic search of 1000 random addition analyses of tree bisection-reconnection (TBR) branch swapping, with all characters being unordered and equally weighted. Under the Corrected Akaike Information Criterion (AICc; Hurvich and Tsai, 1989), the TIM2+G model was selected as the best-fit model for our data set by likelihood ratio tests implemented in jModelTest 0.0.1 (Posada, 2008; Guindon and Gascuel, 2003). Under this model, ML analysis was performed using the program PhyML Version 3.0 (Guindon and Gascuel, 2003). We set the proportion of invariant sites and the shape of the Gamma distribution in PHYML as 0.6590 and 0 estimated by jModelTest, respectively. The base frequency and ratio of transitions/transversions were optimized by the ML criterion in PHYML. To assess the confidences of branches in MP

Table 1 Specimens for molecular systematics analyses.

Species or subspecies	Voucher	Locality	GenBank No.**
Ingroup			
T. hainanensis (JF)	MVZ230352	Jianfengling, Hainan, China	DQ517850
T. hainanensis (DL1)	CIB-20081048	Diaoluo Shan, Hainan, China	#
T. hainanensis (DL2)	CIB-20081049	Diaoluo Shan, Hainan, China	#
T. vietnamensis (QT1)	ROM35330	Quang Thanh, Cao Bang, Vietnam	DQ517856
T. vietnamensis (QT2)	ROM 35364	Quang Thanh, Cao Bang, Vietnam	HM462056
T. asperrimus (JX)	CIB-200807055	Jinxiu, Guangxi, China	#
T. asperrimus (LS)	CIB-70063	Longsheng, Guangxi, China	#
T. notialis	FMNH 271122	Boualapha, Khammouan, Laos	HM462063
T. w. dabienicus (DB1)	HNNU1004 Ⅱ 024	Dabie Mountains, Anhui, China	#
*T. w. dabienicus (*DB2)	HNNU1004 Ⅱ 015	Dabie Mountains, Anhui, China	#
T. w. wenxianensis (PW)	MVZ236632	Pingwu, Sichuan, China	DQ517855
T. w. wenxianensis (WX)	20090527	Wenxian, Gansu, China	#
T. broadoridgus sp. nov.*	CIB-200085	Sangzhi, Hunan, China	#
Outgroup			
T. taliangensis (SM)	CIB-GG200110183	Shimian, Sichuan, China	#
T. taliangensis (LS)	CAS195126	Liangshan, Sichuan, China	DQ517853
T. kweichowensis	MVZ230371	Daguan, Yunnan, China	DQ517851
T. shanjing	MVZ219763	Jingdong Yunnan, China	DQ517852

CIB: Chengdu Institute of Biology, Chinese Academy of Sciences; HNNU: Henan Normal University; ROM: Royal Ontario Museum; FMNH: Field Museum of Nature and History, MVZ: the Museum of Vertebrate Zoology; CAS: California Academy of Sciences.
*: The new species *T. broadoridgus* sp. nov., was previously identified as Sangzhi population of *T. asperrimus* or *T. wenxianensis*.
**: The DNA sequences with # were newly sequenced for this work, and the others were downloaded from GenBank (Weisrock *et al.*, 2006).

and ML trees, we used non-parametric bootstrapping with heuristic searches of 1 000 replicates (Felsenstein, 1985; Felsenstein and Kishino, 1993; Hedges, 1992). Tree topologies with bootstrap values (bs) 70 % or greater were regarded as sufficiently resolved (Huelsenbeck and Hillis, 1993).

Bayesian analyses using the Markov-chain Monte-Carlo technique (MCMC) were conducted for the combined data by using MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003). We used the TrN+G model under the Bayesian Information Criterion (BIC) estimated in jModelTest and initiated two dependent runs, each with four simultaneous MCMC chains, for 1×10^7 generations and sampled trees every 1 000 generations. We determined the burn-in period graphically by using the parameter log-likelihood scores, which were found to be stabilized after 2 500 000 generations within and among the four independent analyses. The first 25 000 trees were discarded as burn-in, and from the remaining trees we obtained a 50% Bayesian consensus tree and posterior probability (PP) values for nodes. The PPs of 95% or greater were considered to be significantly strong support (Leaché and Reeder, 2002; Parra-Olea *et al*., 2004).

Genetic distances among taxa were calculated using Kimura 2-Parameter model in MEGA (Tamura et al., 2007).

3. Results

3.1 Morphological comparison Based on morphological comparisons of the *T. asperrimus* group (Table 2, Figure 2), the Sangzhi population is characterized by the combination of the following morphological characters which differ from other species in the *T. asperrimus* group: a) Dorsal body and tail sre blackish brown without colored spots (Figure 2 A, B); b) tubercles on the dorsum and venter are subequal in size (Figure 2 A, B), and there is no transverse fissure on ventral skin of trunk; c) skull is subequal in length and width (13.6 mm long and 13.4 mm wide) (Figure 5); d) dorsal ridge is comparatively broad and is subequal to eye diameter (Figure 2 H); e) tail height is greater than width of tail at the base; f) no villous genital papillae are found on inner cloacal surface of male during breeding season (Figure 6); g) nodule-like warts present on body sides, bulge and form tubercles; and h) thin transverse striae present between the tubercles on lateral body. These show that the Sangzhi population differs from other species in the *T. asperrimus* group even though they are otherwise morphologically similar to each others.

The Sangzhi population differs from *T. hainanensis* by having the character (c) mentioned above. The head of *T. hainanensis* is wide and large, with the skull being wider than long (1.24 fold; Fei *et al.*, 1984).

It differs from *T. notialis* by possessing the characters

Table 2 Morphological comparison between *T. broadoridgus* sp. nov. and the similar species of the *T. asperrimus* group

Items	*T. asperrimus* 12 ♂	*T. broadoridgus* 30 ♂	*T. wenxianensis* 6 ♂
Snout-vent length (mm)	61.09 ± 2.33 (57.5–65.5)	66.5 ± 3.2 (61.1–72.6)	68.83 ± 4.5 (61.9–77.3)
Dorsal ridge width and eye diameter (mm)	DRW (2.43 ± 0.22) < ED (4.05 ± 0.26)	DRW (4.27 ± 0.47) ≌ ED (4.17 ± 0.2)	DRW (2.31 ± 0.40) < ED (4.16 ± 0.27)
Tail height and width of basal tail (mm)	TH (7.43 ± 1.07) ≌ TW (7.47 ± 0.6)	TH (10.74 ± 0.96) > TW (8.92 ± 0.67)	TH (5.78 ± 1.41) < TW (7.06 ± 1.11)
Villous genital papilla on wall of anus	No villous genital papilla	No villous genital papilla	Many villous genital papillae
Warts on body sides	Rod-like, hockey stick-shaped warts with separated tubercles	Rough, nodular-like warts with transverse striae between continuous tubercles	Band-like, flat warts
Tubercles around anus	Flat tubercles around anus, orange	Projecting tubercles around anus, with wide or narrow orange edge (a few without any)	Projecting tubercles around anus, without orange edge
Head shape and ventral head, and description of neck groove	Head approximately rounded with relatively wide jaw edge ridge from neck groove to mouth corner, prominent oblique cheek	Head approximately trapezoidal no ridge from neck groove to mouth corner, oblique cheek not very prominent	Head approximately oval, neck slightly narrow. No ridge from neck groove to mouth corner; oblique cheek not very prominent

All the measurements (mm) are presented as mean ± SD.

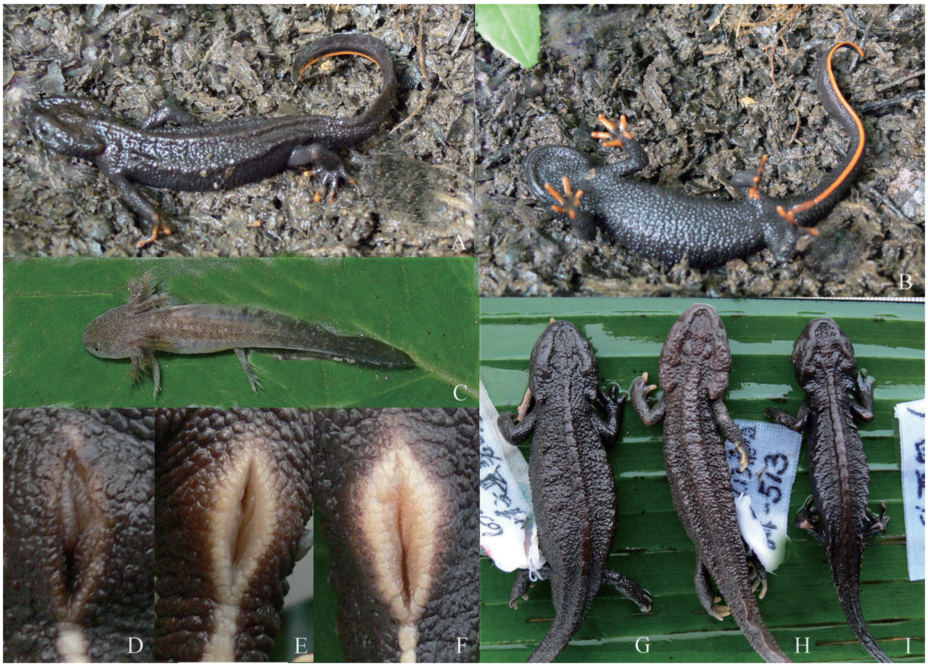

Figure 2 Photos of *T. broadoridgus* sp. nov., and comparisons with *T. wenxianensis* and *T. asperrimus*. Dorsal view (A) and ventral view (B) of *T. broadoridgus* sp. nov. (HNUL 080721A6), and a larva (C) of *T. broadoridgus* sp. nov. Comparison was made at the cloacal region among *T. wenxianensis* (CIB 638164) (D), *T. broadoridgus* (HNUL 840513513) (E), *T. asperrimus* (GXSD 8206-7b) (F), and at the area of dorsal ridges and lateral knob-like warts among *T. wenxianensis* (CIB 638164) (G), *T. broadoridgus* (HNUL 840513513) (H), and *T. asperrimus* (GXSD 8206-7b) (I).

(a, g). *Tylototrton notialis* has orange coloration on the parotids and the rib nodules laterally (Stuart *et al*., 2010).

It differs from *T. vietnamensis* by having the characters (a, b). The dorsal skin of *T. vietnamensis* is smooth and covered with relatively small warts and glands, and its dorsal body is uniformly grayish tan or slightly brownish. The orange rib nodules on lateral body are clearly separate (Böhme *et al*., 2005).

It differs from *T. asperrimus* by having the characters (d, b, g). The width of dorsal ridge of *T. asperrimus* is smaller than the diameter of the eye, and it has hockey stick-shaped warts with separated tubercles. Ventral tubercles range from few to none. There are many fine transverse wrinkles on the ventral skin of the trunk (Table 2, Figure 2) (Fei *et al*., 1984).

It differs from *T. wenxianensis* by having the characters (d, e, f, g), even though they are morphologically similar. The width of the dorsal ridge of *T. wenxianensis* is much smaller than the eye diameter. Tail height is smaller than the width at tail base. There are many villous genital papillae on the inner cloacal surface of male during reproductive season. The band-like, flat warts on body sides are not clearly separate (Table 2, Figure 2) (Fei *et al.*, 1984).

Chen *et al.* (2010) found *Tylototriton wenxianensis dabienicus* ssp. nov. based on morphological and genetic evidence of the specimens collected from the Dabie Mountains. The new subspecies can be distinguished by the combination of the following characteristics: head much longer than wide, limbs a short, and the margin of the cloacal opening orange, though it superficially resembles *T. wenxianensis*. Therefore, the Sangzhi population is also different from *T. wenxianensis dabienicus*.

The basic measurements of the specimens of the Sangzhi population, *T. asperrimus*, and *T. wenxianensis* are shown in Table 3. The results of one-way ANOVA showed that the differences of TOL, TH, TW and DRW, except for ED, are significant among the three forms ($P < 0.05$), but multiple comparisons indicate that the difference of SVL between the Sangzhi population and *T. wenxianensis* is not significant. However, the differences from other comparisons appear to be significant. The results of the KWT showed the differences in the ratios of DRW/ED and TH/TW among the three forms are significant ($P < 0.05$).

3.2 Molecular systematic comparison Aligned ND2 sequences of all 17 samples in this article yielded a total 1035 bps, of which 245 bps were variable, including 195 parsimony informative sites.

All phylogenetic trees derived from BI, MP, and ML using the ND2 sequence data essentially showed the same topologies (Figure 3), indicating that the *T. asperrimus*

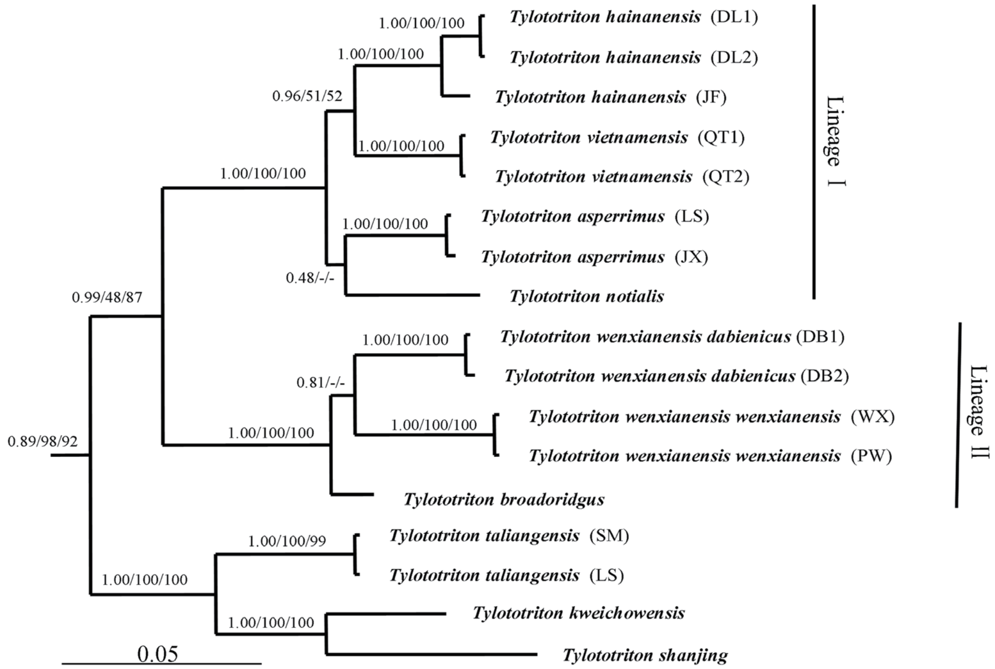
1.00/100/100
Tylototriton hainanensis (DL1)
1.00/100/100
Tylototriton hainanensis (DL2)
0.96/51/52
Tylototriton hainanensis (JF)
1.00/100/100
Tylototriton vietnamensis (QT1)
1.00/100/100
Tylototriton vietnamensis (QT2)
1.00/100/100
Tylototriton asperrimus (LS)
Tylototriton asperrimus (JX)
0.48/-/-
0.99/48/87
Tylototriton notialis
Lineage I
1.00/100/100
Tylototriton wenxianensis dabienicus (DB1)
0.81/-/-
Tylototriton wenxianensis dabienicus (DB2)
1.00/100/100
Tylototriton wenxianensis wenxianensis (WX)
0.89/98/92
1.00/100/100
Tylototriton wenxianensis wenxianensis (PW)
Tylototriton broadoridgus
Lineage II
1.00/100/99
Tylototriton taliangensis (SM)
1.00/100/100
Tylototriton taliangensis (LS)
Tylototriton kweichowensis
1.00/100/100
0.05
Tylototriton shanjing

Figure 3 The phylogenetic relationships among the *T. asperrimus* group inferred from ND2 of mtDNA. The numbers above branches represent bootstrap supports for Bayesian posterior probabilities (BPP), and maximum parsimony (MP) and maximum likelihood (ML).

Table 3 The morphometric differences among *T. broadoridgus* sp. nov., *T. asperrimus* and *T. wenxianensis*. All data are presented as Mean ± SD. One-way analysis of variance (ANOVA) was used to compare the mean differences in the three species. The least significance difference (L.S.D.) was used for multiple comparison.

Measurements	*T. broadoridgus* sp. nov.	*T. asperrimus*	*T. wenxianensis*	One-way ANOVA		*P* values of L.S.D.	
(mm)	(n = 30)	(n = 12)	(n = 6)	*F*	*P* value	A and B	A and C
TOL	126.03 ± 7.79	115.68 ± 6.21	122.71 ± 8.41	9.15	= 0.001	< 0.001	0.079
TH	10.74 ± 0.96	7.43 ± 1.07	5.78 ± 1.41	81.47	< 0.001	< 0.001	< 0.001
TW	8.92 ± 0.67	7.47 ± 0.64	7.06 ± 1 .11	27.29	< 0.001	< 0.001	< 0.001
DRW	4.27 ± 0.47	2.43 ± 0.22	2.31 ± 0.40	115.36	< 0.001	< 0.001	< 0.001
ED	4.17 ± 0.20	4.05 ± 0.27	4.16 ± 0.27	1.34	= 0.272	0.114	0.947

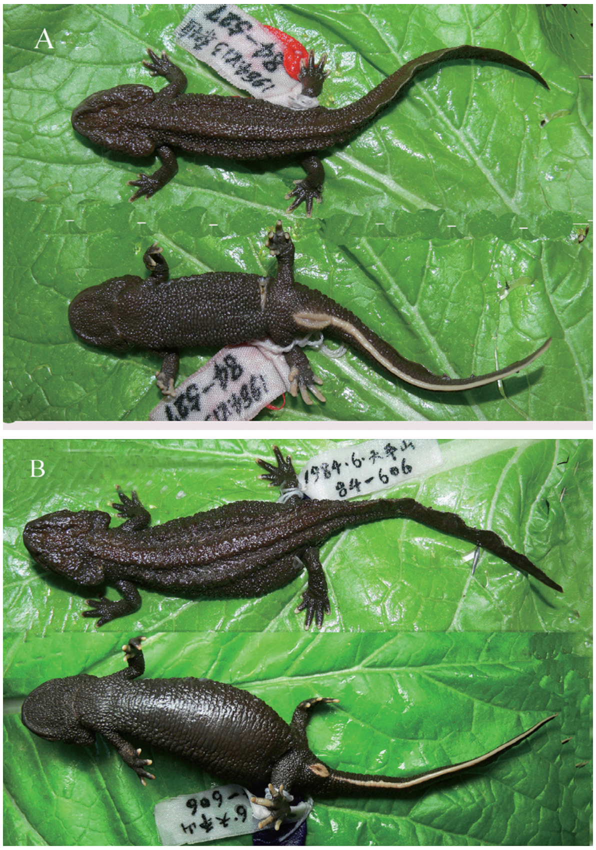

Figure 4 The dorsal and ventral view of the holotype (A) and the allotype (B) of *T. broadoridgus* sp. nov.

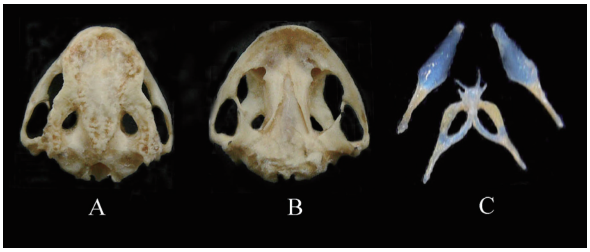

Figure 5 Dorsal view (A) and ventral view (B) of the skull, and the hyoid apparatus (C) of *T. broadoridgus* sp. nov. (HNUL 8405135233, ♂).

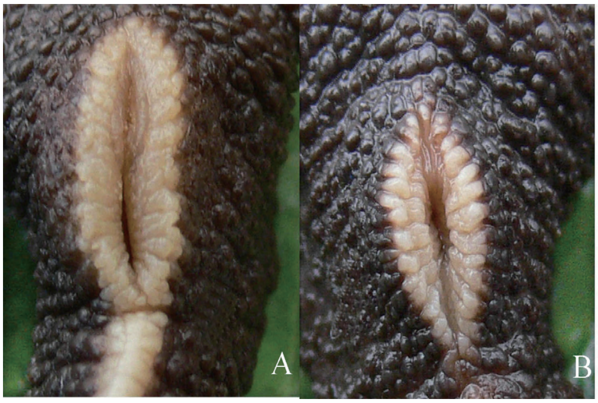

Figure 6 The cloaca of the holotype (A) and the allotype (B) of *T. broadoridgus* sp. nov.

group is monophyletic, corresponding to the subgenus *Yaotriton* (Dubois and Raffaëlli, 2009), and consists of two lineages, that is, *T. vietnamensis, T. asperrimus, T. hainanensis* and *T. notialis*is cluster together as one lineage (Lineage I, BI/MP/ML: 1.00/100/100), while the Sangzhi, Wenxian, Pingwu and Dabie populations of '*T. wenxianensis*' form another lineage (Lineage II, BI/MP/ML: 1.00/100/100). There are three sub-lineages found in the *T.wenxianensis* lineage, the Sangzhi populations, the Dabie population, and Wenxian - Pingwu population, corresponding to the proposed new forms, *T. wenxianensis dabienicus*, and *T. wenxianensis wenxianensis*, and the latter two sub-lineages being sister taxa (Figure 3).

The genetic distances among the 14 populations of the *T. asperrimus* group (Table 4) show that inter-species genetic distances in Lineage I (*T. asperrimus, T. hainanensis, T. notialis* and *T. vietnamensis*) are 0.043–0.053, and the intra-species genetic distances of *T. asperrimus* and *T. hainanensis* are 0.000 and 0.013, respectively. In Lineage II, the genetic distance between the Sangzhi population and *T. wenxianensis wenxianensis* (from Wenxian and Pingwu) is 0.038–0.040, and *T. wenxianensis dabienicus* (from the Dabie Mountains) is 0.034–0.035, while the genetic distance between *T. wenxianensis wenxianensis* and *T. wenxianensis dabienicus* is 0.051–0.052. These genetic distances together with their phylogenetic relationships above indicate that the genetic differentiation of the Sangzhi population has possibly reached a species level differentiation (Stuart *et al*., 2010).

4. Conclusion

Systematics: The results from the morphological and molecular systematic comparisons above reciprocally support that the new form from Sangzhi is more closely related to *T. wenxianensis* than to *T. asperrimus*, and should be recognized as a new species. At the same time, the subspecies *T. wenxianensis dabienicus* should be upgraded as a species, *T. dabienicus*, rather than a subspecies of *T. wenxianensis* on the basis of the molecular systematic comparison. We name the Sangzhi population of "*T. wenxianensis*" as *Tylototriton broadoridgus* sp. nov. in this article.

***Tylototriton broadoridgus* sp. nov.:** (Table 5, Figures 2, 4, 5, 6).

Holotype: HNUL 840513527, male adult, was collected by Youhui SHEN from Liaoyewan in the Tianping Mountains (29°49′N, 110°9′E) in Sangzhi County, Hunan

Table 4 Genetic distances among the 14 populations of the *T. asperrimus* group

	Species or subspecies	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*T. hainanensis* (DL1)																
2	*T. hainanensis* (DL2)	0															
3	*T. hainanensis* (JF)	0.013	0.013														
4	*T. notialis*	0.05	0.05	0.049													
5	*T. vietnamensis* (QT2)	0.044	0.044	0.043	0.05												
6	*T. asperrimus* (JX)	0.052	0.052	0.051	0.05	0.046											
7	*T. asperrimus* (LS)	0.053	0.053	0.052	0.051	0.047	0.001										
8	*T. vietnamensis* (QT1)	0.045	0.045	0.044	0.052	0.001	0.047	0.048									
9	*T. wenxianensis* (DB1)	0.097	0.097	0.098	0.1	0.103	0.1	0.099	0.104								
10	*T. wenxianensis* (DB2)	0.095	0.095	0.097	0.099	0.102	0.099	0.098	0.103	0.001							
11	*T. wenxianensis* (PW)	0.095	0.095	0.096	0.096	0.103	0.094	0.095	0.104	0.052	0.051						
12	*T. wenxianensis* (WX)	0.095	0.095	0.096	0.096	0.103	0.094	0.095	0.104	0.052	0.051	0.002					
13	*T. broadoridgus* sp. nov.*	0.078	0.078	0.082	0.086	0.086	0.083	0.084	0.087	0.035	0.034	0.038	0.04				
14	*T. taliangensis* (SM)	0.093	0.093	0.094	0.102	0.109	0.1	0.101	0.11	0.1	0.099	0.094	0.094	0.095			
15	*T. taliangensis* (LS)	0.093	0.093	0.094	0.102	0.109	0.1	0.101	0.11	0.1	0.099	0.094	0.094	0.095	0		
16	*T. kweichowensis*	0.09	0.09	0.091	0.106	0.103	0.107	0.109	0.104	0.109	0.108	0.105	0.105	0.097	0.067	0.067	
17	*T. shanjing*	0.107	0.107	0.108	0.12	0.121	0.121	0.122	0.122	0.121	0.119	0.106	0.106	0.106	0.08	0.08	0.06

*: The new species *T. broadoridgus* sp. nov. was identified as *T. asperrimus* or *T. wenxianensis* in the past.

on May 13, 1984.

Allotype: HNUL 841000606, female adult, was collected by Youhui SHEN from the same place of the holotype on June 6, 1984,

Paratype: 34 specimens (♂), HNUL 88050001, 840513506–840513508, 080721A6, 840513513–840513524, 840513528–840513533, 840600601–840600607, 820800806, 820800844, 820800859, 820800862; and 3 specimens (♀), HNUL 88050002, 88050003 and 840513523, were collected by Youhui SHEN from the same place of the holotype in August, 1982, May, June, October, 1984, May, 1988, and July, 2008.

The type specimens are deposited in the Animal Museum of the College of Life Sciences, Hunan Normal University in Hunan, China.

Diagnosis: The new species is close to *T. wenxianensis*, but differs from it by having the following features: 1) Comparatively broad dorsal ridge [Its width is almost equal to eye diameter ($P < 0.001$)] (Figure 2 H); 2) tail height greater than the width at tail base ($P < 0.~001$); 3) males without villous genital papilla on the inner surface of anus; 4) nodule-like warts on the lateral body rough and relatively bulge, forming tubercles, between which thin and transverse striae present (Figure 2 H).

Description of the holotype: Total length of the holotype is 127 mm (Other measurements are shown in Table 5). Head compressed, longer than wide, broader than body width, (HL 19.0 mm, HW 17.9 mm); Snout short, truncate in ventral view rounded in profile, exceeding beyond lower jaw; the rostrum narrower than head width; the bony ridges on the head notable, extending from the rostral side, and through the interior side of the upper eyelid to occiput. The two triangular bony ridges on the occiput point laterally and form a “V” shape, distinctly being not connected with the dorsal ridge of body (Figure 2 H). The inter-nasal space is smaller than the inter-orbital space; eyes are relatively large (ED 4.4 mm), protrude from the dorsolateral portion of the head and have circular eyelids. The oral fissure is flat, straight and extends a distance greater than half of the length of the head. The joint of jaw articulation lies posterior to the caudal margin of the eyes. Fine teeth present on the edge of jaw, vomerine teeth form a“∧” shape (Figure 5 b) and extend posteriorly behind the orbit to an approximate length of 7–8 teeth. The tongue is oval and slightly concave in the middle, nearly entirely fixed at bottom but free at both lateral edges. The neck is rounded and thick, with distinct neck groove.

Body is stout with a pentagonal cross-section; dorsal ridge, extending from the neck to the base of the tail (Figure 2 H), is rough and broad, as almost equal to the diameter of the eye (ED 4.4 mm). Two sides of the ridge are slightly concave. Each lateral corner of the dorsal ridge consists of a row of rough and large nodular tubercles, and fine transverse striae present between every two tubercles. The ventral area of the body is relatively flat.

Four limbs are relatively slender, and hind limbs are slightly longer than forelimbs. The distal tips of limbs overlap slightly when fore and hind limbs are adpressed along the trunk. The relative length of fingers is: III > II >

Table 5 Measurements of the adult specimens of *T. broadoridgus* sp. nov.

Measurements	Holotype ♂	%	Allotype ♀	%	Paratype 29 ♂		%	Paratype 3 ♀		%
(mm)	HNUL 840513527		HNUL 841000606		Means ± SD	Range		Means ± SD	Range	
TOL	127	192.4	162.5	180	125.7 ± 7.5	(110.4–140.3)	189	149.03 ± 12.1	(138.9–162.5)	178.9
SVL	66		90.4		66.5 ± 3.2	(61.1–72.6)		83.3 ± 6.1	(79.5–90.4)	
HL	19	28.8	21.8	24.1	18.0 ± 0.9	(16.0–20.2)	27	20.5 ± 1.2	(19.4–20.4)	24.6
HW	17.9	27.1	20.8	23	16.5 ± 1.0	(15.0–18.4)	24.7	19.6 ± 1.8	(16.8–20.8)	23.5
HH	8.9	13.5	9.8	10.8	7.8 ± 0.6	(6.6–8.8)	11.8	9.23 ± 0.7	(8.5–9.8)	11.1
SL	6.3	9.05	7	7.7	6.2 ± 0.4	(5.4–7.0)	9.4	6.4 ± 0.5	(6.1–7.0)	7.7
ED	4.4	6.6	4.7	5.2	4.2 ± 0.2	(3.4-4.5)	6	4.3 ± 0.3	(3.9–4.7)	5.5
INS	4.8	7.3	5.8	6.4	5.0 ± 0.3	(4.5–5.5)	7.5	4.7 ± 0.9	(4.2–5.8)	5.6
IOS	6.8	10.3	8.8	9.7	7.0 ± 0.4	(6.2–7.7)	10.5	8.3 ± 0.5	(7.9–8.8)	9.9
FLL	21.5	32.6	25.8	28.5	20.9 ± 0.9	(19.7–22.4)	31.5	23.07 ± 2.7	(20.4–25.8)	27.7
HLL	22	33.3	26.2	29	22.1 ± 0.9	(20.2–23.7)	33.2	25.3 ± 1.6	(23.4–26.2)	30.3
AGS	27.9	41.8	44.6	49.3	28.9 ± 2.3	(26.1–34.6)	43.4	39.7 ± 4.7	(35.2–44.6)	47.6
TL	60.8	92.1	72.1	79.8	59.8 ± 4.4	(47.1–65.4)	90	64.7 ± 6.8	(58.8–72.1)	77.6
TH	11.6	17.6	10.6	11.7	10.8 ± 0.9	(8.7–11.9)	16.3	9.8 ± 0.72	(9.2–10.6)	11.7
TW	9.6	14.5	9.9	11	8.6 ± 0.5	(7.9–9.5)	13	9.37 ± 0.5	(8.8–9.9)	11.2

% is the ratio of each measurement to SVL.

IV > I, and relative length of toes: III > IV > II > V >I. All digits are slightly flat with obtuse ends and none webbed. The tail length is less than snout-vent length. Tail is notably compressed laterally, with relatively strong tail muscles. The dorsal fin fold of the tail, starting from the tail base, is distinctly thin and high; while the ventral fin fold of tail, starting from posterior to the cloaca, is thick and short. The tail height is greater than the width at the tail base, and the distal tail tip is rounded. The cloaca is long and narrow, and the cloacal region is slightly bulbous.

Skin is extremely rough. Body is covered with tubercles and warts, while the labial margin, distal limbs, ventral limbs and ventral edge of tail are smooth. On the lateral dorsum of the body, tubercles and warts are big and closely arranged, appearing to form lines that extend from shoulder to tail base. Unlike the dorsal tubercles, the ventral tubercles and warts are relatively flat, and are either transversely arranged or have transverse striae.

Color in life: The entire specimen is almost black or blackish-brown, but the ventral tail fin folds, peripheral area of cloaca, distal digit ends, and ventral digits are orange. The orange region between the ventral fin fold of tail and the peripheral area of cloaca is mostly connected (The orange coloration fades to a whitish hue in gravid females).

Measurements: Measurements of the holotype, allotype and paratype are present in Table 5. For the paratype, the average SVL of female is 83.3 mm while that of male 66.5 mm. The female has a comparatively long auxiliary spacing which is about 39.7 mm on average and is 47.6% of the SVL, while the male's auxiliary spacing is only about 28.9 mm long and is 43.4% of the SVL. On contrast, the tail of the female is comparatively short, about 64.7 mm and 77.63% of the SVL, while the male's tail approximately 59.8 mm and 90.0% of the SVL. The tail height of the male is greater than that of female in comparison to its body length (Table 5).

Secondary sexual characteristics: The female body is relatively elongate and robust, which becomes more prominent in the abdomen during breeding season. The female has no labial fold and its inner cloacal wall has protuberances, which are similar to the tubercles around peripheral cloacal region and protrude radically from cloaca to the periphery. The male has labial folds, and its cloaca is often closed and has the protuberances, which are the same as those of female, but has no villous genital papilla on inner cloacal wall (Figure 2 E). The cloacal region is slightly bulbous, and this feature becomes more evident during breeding season in both male and female.

Skull: The bony skull of *T. broadoridgus* is extremely rough and covered with bony tubercles (Figure 5 A). The lateral portion of cranium features prominent bony ridges. The length (13.6 mm) and width (13.4mm) of skull are approximately equal, with 4.9 mm in height. The otic ramus of the squamosal and the occipital condyles are approximately adjacent along the posterior portion of skull. The rows of the vomerine teeth make a "∧" shape, with the apex pointing toward the rostrum (Figure 5 B). Most elements of the skull appear to be completely fused, so it is difficult to find the delimitations of sutures. The front part of the pterygoid bone is connected to a long maxilla bone.

Hyoid apparatus (Figure 5 C)**:** There is a pair of ceratohyal cartilage and epihyal bone on both sides, and the copula (or basibranchial) is partially ossified, though it is largely formed by cartilage. Anterolaterally, the copula bears two pairs of horns, termed as anterior radials (the interior ones) and posterior radials (the lateral ones), and the anterior radials are longer than the posterior radials. The cartilage of the first pair of ceratohyal is ossified, but thatof the second pair of ceratohyal not ossified (in blue color in Figure 5C). The pair of epibranchial bones remain and are connected to the exterior area of a pair of ceratohyal.

Larvae: Larvae were collected from the Tianping Mountains in Sangzhi on June 12, 1986, and were then estimated to be 1 to 1.5 months old after hatching with respect to breeding season.

The larval body is slender and 33.8 mm in total length. The limbs appear to be quite weak, with locomotion primarily occurring through tail movement. The larval head is short with relatively large eyes and three pairs of external gills having obtuse anterior ends. The head is wide and flat, while the body and tail are laterally compressed. Costal grooves for muscles of larvae are readily observable, and forelimbs and hindlimbs are clearly developed then. Skin is smooth and the ventral side of tail is whitish with a few scattered black or blackish-brown spots on the dorsal side of tail. The dorsal fin fold rises from the area between posterior head and anterior body. The dorsal fin fold is comparatively tall, and the short, thick ventral fin fold of tail extends from anus to tail end.

When the larvae reach approximately 57.8 mm long, the external gills appear more prominent, and lateral lines consisting of tubercles begin to form (Figure 2 C). The dorsal fin fold of tail starts from the middle of body. The body itself is completely black, except for the orange ventral fin fold and digit ends. When larvae are 62 mm long, the external gills begin to atrophy, dorsal ridge becomes more prominent, and the dorsal fin fold of tail moves towards the posterior part of body.

Generally, metamorphosis is completed before winter, though some larvae with external gills were collected in late March. Larvae generally survive the winter season under dry branches, fallen leaves, or by hiding themselves underground, and a few of them survive the winter by remaining at bottom of pond.

Ecological habits: This new species lives in a mountainous area at an elevation from 1000 m to 1600 m, in bamboo groves or where the land is covered with thick, dry branches and fallen leaves. The species is mainly terrestrial, but enters still pools in breeding season, being most attracted to where the humus at the bottom of the pond is rich and the water murky. In early May each year, the young males firstly enter their breeding field, and then the adult females come in. The adult females are sexually matured after the males. Adult salamanders leave ponds after breeding to live in forest, taking refuge under dry branches and fallen leaves, though some have been observed to remain in or near water. As late as July, the adult males can still be active at pond floor, with the activity typically subsiding in November.

Distribution: The new species is collected in the Tianping Mountains in Sangzhi, northwestern Hunan, China.

Etymology: The new species is named for its broad dorsal ridge.

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References

Böhme W., Schottler T., Nguyen T. Q., Kohler J. 2005. A new species of salamander, genus *Tylototriton* (Urodela: Salamandridae) from northern Vietnam. Salamandra, 41: 215–220

Chang M. L. Y. 1936. Contribution à l'étude Morphologique. Biologique et Systématique des Amphibiems Urodèles de la Chin. Paris: Librairine Picart, 150 pp

Chen X. H., Wang X. W., Tao J. 2010. A new subspecies of genus *Tylototriton* from China (Cudata, Salamandridae), Acta Zootaxonom Sin, 35(3): 666–670

Dubois A., Raffaëlli J. 2009. A new ergotaxonomy of the family Salamandridae Goldfuss, 1820 (Amphibia, Urodela). Alytes, 26: 1–85

Fang P. W., Chang M. L. Y.1932. Notes on *Tylototriton kweichouensis* Unterstein with synopsis to species. Sinensia, 2(9): 111–122

Fei L., Hu S. Q., Ye C. Y., Huang Y. Z. 2006. Fauna Sinica. Amphibia,Vol. I. Beijing: Science Press, 1–471 (In Chinese)

Fei L., Ye C. Y., Jiang J. P. 2010. Colored Atlas of Chinese Amphibians. Chengdu. China: Sichuan Publishing House of Science and Technology, 1–519 (In Chinese)

Fei L., Ye C. Y., Yang R. S. 1984. A new species and new subspecies of the genus *Tylototriton* (Caudata: Salamandridae.) Acta Zool Sin, 30(1): 85–91 (In Chinese)

Fei L., Ye C. Y., Huang Y. Z. 1990. The Key to Chinese Amphibian. Chongqing: Chongqing Branch of Science and Technology Literature Publishing House, 1–364 (In Chinese)

Fei L., Ye C. Y., Huang Y. Z., Jiang J. P., Xie F. 2005. An Illustrated Key to Chinese Amphibians. Chengdu: Sichuan Publishing House of Science and Technology, 1–340 (In Chinese)

Felsenstein J., Kishino H. 1993. Is there something wrong with the bootstrap on phylogeny? A reply to Hillis and Bull. Syst Zool, 42: 193–200.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39: 783–791

Guindon S., Gascuel O. 2003.A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol, 52: 696–704

Hedges S. B. 1992. The number of replications needed for accurate estimation of the bootstrap P-value in phylogenetic studies. Mol Biol Evol, 9: 366–369

Hu S. Q., Djao Er-mie (Zhao E. M.), Liu C. C. 1973. A survey of amphibians and reptiles Kweichow (= Guizhou) Province, including a herpetofaunal analysis. Acta Zool Sin, 19(2): 149–178 (In Chinese)

Hu S. Q., Ye C. Y., Fei L. 1977. Systematic Key to Amphibians of China. Beijing: Science Press, 1–93 (In Chinese)

Huelsenbeck J. P., Hillis D. M. 1993. Success of phylogenetic methods in the four-taxon case. Syst Biol, 42: 247–264

Hurvich C. M., Tsai C. L. 1989. Regression and time series model selection in small samples. Biometrika, 76: 297–307

Leaché A. D., Reeder T. W. 2002. Molecular systematics of the eastern fence lizard (*Sceloporusundulatus*): A comparison of parsimony, likelihood, and Bayesian approaches. Syst Biol, 51: 44–68

Parra-Olea G., García-París M., Wake D. B. 2004. Molecular diversification of salamanders of the tropical American genus *Bolitoglossa* (Caudata: Plethodontidae) and its evolutionary and biogeographical implications. Biol J Linn Soc, 81: 325–346

Posada D. 2008. jModelTest: Phylogenetic model averaging. Mol Biol Evol, 25: 1253–1255

Ronquist F. R., Huelsenbeck J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19: 1572–1574

Shen Y. H. 1989. A survey of the tailed amphibians in Hunan Province. Chin Wildlife, (6): 77–84 (In Chinese)

Stuart B., Phimmachak S., Niane S., Robichaud W. G. 2010. A new species in the *Tylototriton asperrimus* group (Caudata: Salsmsndridae) from central Laos. Zootaxa, 2650: 19–32

Swofford D. L. 2002. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods). Sunderland, MA: Sinauer

Tamura K., Dudley J., Nei M., Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol, 24: 1596–1599

Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F., Higgins D. G. 1997. The Clustal X windows interface: Flexible strategies for multiple sequences alignment aided by quality analysis tools. Nucl Acids Res, 25: 4876–4882

Tian W. S., Jiang Y. M. 1986. Identification Manual of Chinese Amphibians and Reptiles. Beijing, China: Science Press, 1–164 (In Chinese)

Wang B., Jiang J. P., Xie F., Chen X. H., Dubois A., Liang G., Wagner S. 2009. Molecular phylogeny and genetic identification of populations of two species of *Feirana* frogs (Amphibia: Anura, Ranidae, Dicroglossinae, Paini) endemic to China. Zool Sci, 26: 500–509

Weisrock D. W., Papenfuss T. J., Macey J. R., Litvinchuk S. N., Polymeni R., Ugurtas I. H., Zhao E. M., Jowkar H., Larson A. 2006. A molecular assessment of phylogenetic relationships and lineage accumulation rates within the family Salamandridae (Amphibia, Caudata). Mol Phylogenet Evol, 41: 368–383